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1: Cancer Res. 1998 May 1;58(9):1965-71. Related Articles, Links

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Comment in:
- Cancer Res. 1999 Sep 1;59(17):4471-2.

DNA vaccines encoding full-length or truncated Neu induce protective immunity against Neu-expressing mammary tumors.

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Chen Y, Hu D, Eling DJ, Robbins J, Kipps TJ.

University of California at San Diego Human Gene Therapy Program, Department of Medicine, University of California at San Diego School of Medicine, La Jolla 92093-0663, USA.

We generated DNA expression vectors encoding the full-length neu cDNA (designated pNeuN), the neu extracellular domain (pNeuE), or the neu extracellular and transmembrane domains (pNeuTM). The 293 cells transfected with pNeuN or pNeuTM expressed the neu extracellular domain on the surface membrane, whereas 293 cells transfected with pNeuE secreted the extracellular domain of neu into the culture supernatant. We examined whether i.m. injection of either of these plasmids could induce protective immunity in FVB/N mice against the adoptive transfer of Tgl-1 cells, a neu-expressing tumor cell line generated from a mouse mammary tumor that spontaneously arose in a FVB/N neu-transgenic mouse. The i.m. injection of pNeuTM or pNeuE, and to a lesser extent pNeuN, induced protective immunity against a subsequent challenge with Tgl-1 cells in FVB/N mice. In addition, the coinjection of a plasmid encoding interleukin-2 (designated pIL-2) augmented the efficacy of each of the pNeu plasmids for inducing protective immunity. The plasmid pNeuTM seemed to be the most effective for inducing anti-neu antibodies. However, the generation of detectable anti-neu antibodies in response to any one of these pNeu plasmids was not enhanced by coinjection of pIL-2 and was not required for protective immunity against Tgl-1 cells. These studies demonstrate that DNA expression vectors encoding soluble or membrane-bound forms of neu lacking the cytoplasmic kinase domain can be effective in inducing protective antitumor immunity.

PMID: 9581840 [PubMed - indexed for MEDLINE]

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1: Cancer Res. 1998 Nov 1;58(21):4902-8. Related Articles, Links

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Immunization with a peptide epitope (p369-377) from HER-2/neu leads to peptide-specific cytotoxic T lymphocytes that fail to recognize HER-2/neu+ tumors.

Zaks TZ, Rosenberg SA.

Surgery Branch, National Cancer Institute, NIH, Bethesda, Maryland 20892-1502, USA. zakst@nih.gov

The oncogene HER-2/neu is genetically amplified and overexpressed in a large number of human adenocarcinomas and has been implicated in the tumorigenic phenotype. Although it is a nonmutated self-protein, it is barely detectable in adult tissues, and immune responses toward it have been described in a number of patients. It is, thus, an attractive candidate antigen for the immunotherapy of cancer patients. HLA-A2+ patients with metastatic breast, ovarian, or colorectal adenocarcinomas that overexpressed HER-2/neu were immunized with the HLA-A2-binding epitope p369-377 (p369). Patients were treated by repeated immunization with 1 mg of p369 in Freund's incomplete adjuvant every 3 weeks. Peripheral blood mononuclear cells were collected prior to immunization and following two and four immunizations and were stimulated in vitro with peptide and assayed for peptide and tumor recognition. In three of four patients, peptide-specific CTLs were detected in post- but not preimmunization blood. These CTLs recognized peptide-pulsed target cells at peptide concentrations of > or =1 ng/ml yet failed to react with a panel of HLA-A2+ HER-2/neu+ tumor lines. In addition, infecting HLA-A2+ cells with recombinant vaccinia virus encoding HER-2/neu or up-regulating HLA-A2 with IFN-gamma in HER-2/neu+ cells also failed to confer reactivity by p369-reactive T-cells. A T-cell response to the HLA-A2 binding epitope p369 can be easily generated by immunizing patients with peptide in Freund's incomplete adjuvant. However, the CTLs failed to react with HER-2/neu+ tumor cells. Further studies are needed to determine whether and how HER-2 might serve as an antigen for tumor immunotherapy.

PMID: 9809997 [PubMed - indexed for MEDLINE]

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1: Surgery. 1997 Aug;122(2):235-41; discussion 241-2. Related Articles, Links

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Generation of peptide-specific cytotoxic T lymphocytes using allogeneic dendritic cells capable of lysing human pancreatic cancer cells.

Peiper M, Goedegebuure PS, Eberlein TJ.

Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Mass. 02115, USA.

BACKGROUND: Dendritic cells (DCs) are potent antigen presenting cells (APCs), able to efficiently induce primary T cell-mediated responses to foreign antigens. In earlier studies we were able to identify a histocompatibility antigen (HLA)-A 2-restricted nine amino acid peptide (GP2, peptide 654-662) from the transmembrane portion of the protooncogene HER2/neu as a tumor-associated antigen (TAA) in human pancreatic cancer. METHODS: Peripheral blood mononuclear cells (PBMCs) of HLA-A2+ and HLA-A2 healthy volunteers were isolated. PBMCs were grown with initial anti-CD3, low-dose interleukin-2 (IL-2), and peptide-pulsed DC stimulation. T-cell lines were analyzed in functional studies. RESULTS: After 4 weeks, T-cell cultures were more than 50% CD8+. All peptide-pulsed T cells significantly lysed APC pulsed with the immunizing antigen in an HLA-A2 restricted fashion. Furthermore, HLA-A2+,HER2/neu+ human pancreatic cancer cells were lysed significantly higher than HLA-A2 HER2/neu+ pancreatic cancer cells. Transfection of an HLA-A2 pancreatic cancer cell line with the HLA-A2 gene resulted in a significantly higher lysis of the transfected cell line compared to the wild type. In HLA-A2+ pancreatic cancer targets, specific lysis was HLA-A2 restricted. CONCLUSION: The ability to use DCs for presentation of either tumor or peptide antigen in an HLA-restricted fashion to stimulate T-cell proliferation, as well as cytotoxicity, demonstrates the potential of this technology for future development of a pancreatic cancer vaccine.

PMID: 9288128 [PubMed - indexed for MEDLINE]

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1: J Immunol. 1994 Nov 15;153(10):4775-87. Related Articles, Links

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Idiotype-cytokine fusion proteins as cancer vaccines. Relative efficacy of IL-2, IL-4, and granulocyte-macrophage colony-stimulating factor.

Chen TT, Tao MH, Levy R.

Department of Medicine, School of Medicine, Stanford University, CA 94305.

Idiotypic determinants, antigenic sites expressed on the variable region of Ig molecules of malignant B cells, represent tumor-specific Ags but are weak immunogens. We have previously shown that the immunogenicity can be dramatically increased by fusing tumor Id to granulocyte macrophage (GM)-CSF. Here, we demonstrate that fusion proteins with IL-2 or IL-4 can also be highly immunogenic. Co-immunization of these fusion proteins with another Id demonstrated the importance of physical linkage between the cytokine and relevant Ag for this enhancement. All three fusion proteins are capable of eliciting significant levels of specific Abs against the Id without the use of carrier proteins or adjuvants, although the GM-CSF fusion protein appeared to be unique in its ability to induce higher titers of anti-Id Abs in the primary response. Furthermore, the Id-IL-2 fusion protein induced high titers of IgG2a and IgG3 anti-Id Abs, whereas the Id-IL-4 and Id-GM-CSF fusion proteins did not. Despite the differences, tumor protection was comparable in all mice having significant titers of anti-Id Abs, regardless of the fusion protein used. We concluded that Id-cytokine fusion proteins are potent immunogens that can elicit significant antitumor immunity. The general approach of fusing a cytokine to a potential Ag may be applicable to the design of vaccines for immunotherapy of other types of tumors as well as for other pathogens and disease states.

PMID: 7525715 [PubMed - indexed for MEDLINE]

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L6: Entry 10 of 15 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210662 B1
TITLE: Immunostimulatory composition

Brief Summary Text (4):
The immune response of the mammalian immune system is generally divided into two general types: humoral immunity, mediated largely by circulating antibodies, and cellular immunity mediated by various forms of T-cells. Generally, extracellular antigens stimulate a humoral response, while intracellular antigens such as viruses, stimulate a cellular response.

Brief Summary Text (23):
In further related aspects, the invention also includes expression vectors and expression systems for producing the above-described immunostimulatory fusion proteins, as well as substantially purified nucleic acid molecules that encode such fusion proteins. In preferred embodiments, the nucleic acid molecules code for fusion proteins consisting essentially of GM-CSF and prostatic acid phosphatase or of GM-CSF and Her2.

Drawing Description Text (9):
FIG. 8 shows the nucleic acid (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences of a GM-CSF-Her2 fusion protein in accordance with the present invention.

Detailed Description Text (32):
Fusion proteins constructed to incorporate oncogene product antigens are exemplified by incorporation of the oncogene product Her2. Her2 is a growth factor receptor belonging to the EGF-R family of receptors. It is overexpressed by breast cancer cells, ovarian cancer cells and a variety of other cancer cells. The cDNA coding for the extracellular domain of Her2 is cloned from a breast cancer cell line and fused to the GM-CSF CDNA, essentially as detailed for PAP-GM-CSF, above.

Detailed Description Text (33):
Production of the soluble protein can be verified using Her2-specific monoclonal antibodies in an ELISA test, according to methods well-known in the art. The fusion protein the includes the sequences for the extracellular domain (amino acids 1-652) of Her2 (GenBank) and GM-CSF (FIG. 8). In this particular fusion protein the two proteins are linked by a leucine/glutamic acid linker which is generated by inserting a XhoI site. Other oncogene product antigens are similarly incorporated into fusion proteins according to the methods described herein, using published sequences. In addition, other antigens, such as viral antigens, may be part of a fusion construct, according to the methods described herein.

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L9: Entry 9 of 14 File: USPT Apr 3, 2001

US-PAT-NO: 6210662
DOCUMENT-IDENTIFIER: US 6210662 B1

TITLE: Immunostimulatory composition

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laus; Reiner	San Carlos	CA		
Ruegg; Curtis Landon	San Carlos	CA		
Wu; Hongyu	Palo Alto	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Dendreon Corporation	Seattle	WA			02

APPL-NO: 09/ 344195 [PALM]
DATE FILED: June 24, 1999

PARENT-CASE:
This application is a continuation of U.S. patent application Ser. No. 09/146,283, filed Sep. 3, 1998, now granted as U.S. Pat. No. 5,976,546, which is a divisional of U.S. patent application Ser. No. 08/579,823, filed Dec. 28, 1995 now granted as U.S. Pat. No. 6,080,409, both of which are herein incorporated by reference.

INT-CL: [07] A01 N 63/00, A01 N 65/00, C12 N 5/00, C12 N 5/08

US-CL-ISSUED: 424/93.1; 435/325, 435/366, 435/372, 435/372.3
US-CL-CURRENT: 424/93.1; 435/325, 435/366, 435/372, 435/372.3

FIELD-OF-SEARCH: 424/93.1, 435/325, 435/366, 435/372, 435/372.3

PRIOR-ART-DISCLOSED:

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☐	5019384	May 1991	Gefter et al.	
☐	5078996	January 1992	Colon, III et al.	
☐	5194254	March 1993	Barber et al.	
☐	5616477	April 1997	Price	
☐	5677171	October 1997	Hudziak et al.	

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FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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WO 95/21862	August 1995	WO	
WO 95/23814	September 1995	WO	
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ART-UNIT: 165

PRIMARY-EXAMINER: Navarro; Albert

ATTY-AGENT-FIRM: Judge; Linda R. Iota Pi Law Group

ABSTRACT:

Disclosed are therapeutic compositions and methods for inducing cytotoxic T cell responses in vitro and in vivo. The therapeutic compositions consist of antigen presenting cells activated by contact with a polypeptide complex constructed by joining together a dendritic cell-binding protein and a polypeptide antigen. Also disclosed are expression vectors and systems for producing the polypeptide complexes.

3 Claims, 9 Drawing figures

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L9: Entry 9 of 14 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210662 B1
TITLE: Immunostimulatory composition

Brief Summary Text (14):
These limitations are overcome by the discovery encompassed by the present invention. It is the discovery of the present invention that a T-cell response, and specifically, an MHC-Class I mediated T-cell response, can be stimulated by an isolated or soluble protein, when it is presented to the immune system as part of a complex with a dendritic cell binding protein, and more particularly GM-CSF. It is the further discovery of the present invention that such a response can be stimulated in vitro. As discussed above, in vitro stimulation of such a response has not previously been demonstrated using a full-length soluble antigen. The present invention provides for induction by isolated or soluble proteins of cellular immunity in vitro by presenting a specific antigen to an antigen presenting cell (APC), such as a dendritic cell, as part of an immunogenic fusion protein.

Brief Summary Text (15):
An important aspect of the present invention is the choice of fusion partner protein, a dendritic cell binding protein, such as granulocyte-macrophage colony stimulating protein (GM-CSF). Without relying on any particular mechanistic theory, it is believed that the protein antigen is transported over the plasma membrane of the APC in a receptor mediated non-disruptive way. It is further believed that the dendritic cell binding portion of the fusion protein serves to preserve the viability and functionality of the APC.

Detailed Description Text (39):
The use as APC's of dendritic cells stimulated by GM-CSF fusion antigens yields superior results to other approaches such as peptide pulsed dendritic cells. It is known that dendritic cells pulsed with 8- 11-mer peptides induce immunity that is directed to a single T-cell epitope. Proteins incorporated into liposomes or delivered by osmotic shock induce reactivity towards multiple T-cell epitopes; however, this process is relatively ineffective due to the inherent toxicity of these treatments to dendritic cells. GM-CSF fusion antigens of the present invention, on the other hand, induce immunity towards multiple epitopes and preserve and enhance at the same time viability and function of the dendritic cell.

Detailed Description Text (41):
In experiments carried out in support of the present invention, the fusion protein consisting of PAP and GM-CSF described in the previous section was used for in vitro introduction to dendritic cells and subsequent activation of cytolytic T cells, as detailed in Example 4. Briefly, HLA-A2.1 positive PBMNC were isolated by standard methods and primed with the fusion protein for 2-5 days. The cell mixture was depleted of CD4' T-cells, separated into high and low density fractions, and the separate cultures were restimulated weekly with autologous PAP GM-CSF pulsed APC's. Lytic potential of the T-cells present in the fractions was assessed using a standard chromium release assay using an HLA-A2.1-transgenic prostate carcinoma cell line as target. This novel cell line was constructed according to the methods detailed in Example 3 herein and is useful in screening and analysis of HLA class I restricted cytotoxic T-lymphocytes.

Detailed Description Text (48):
In practice, dendritic cells are isolated from an individual, using known methods, or preferably, as described in Example 5. The dendritic cells (or other APC's) are mixed

with 10 ng/ml equivalent of GM-CSF fusion antigen, as described in Example 4. The cell preparation may then be depleted of CD4' T-cells by solid phase immunoadsorption and further fractionated to enrich for cells having cytolytic activity. Doses of about 10.sup.7 cells are then administered to the subject by intravenous or central injection according to established procedures (e.g., infusion over 30 to 60 minutes). The responsiveness of the subject to this treatment is measured by monitoring the induction of a cytolytic T-cell response, a helper T-cell response and antibody response towards the antigen in peripheral blood mononuclear cells by methods well known in the art.

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L22: Entry 24 of 29 File: USPT Apr 9, 2002

DOCUMENT-IDENTIFIER: US 6368796 B1
**** See image for Certificate of Correction ****
TITLE: Methods of detection and treatment of breast cancer

Detailed Description Text (86):
ErbB-2 functions as a co-receptor for growth-regulatory molecules, including neuregulins. Replacement of the extracellular domain of ErbB-2 by the ligand biding domain of the receptor for EGF allows heterologous stimulation of the ErbB-2, which has been successfully exploited in signal transduction studies (Ben-Levy, R., et al., EMBO J., 13;3302-3311 (1994). The transforming protein of ErbB-2, which contains a Glutamine residue (Glu.sup.664) instead of a Valine (VAL.sup.664) residue, is a constitutively active receptor permanently coupled to signaling pathways. To confirm that the association of CHK with ErbB-2 is mediated by the intracellular domain of ErbB-2 and not by other members of the ErbB-2 family, chimeric proteins that include the extracellular domain of the EGF receptor and the transmembrane and cytoplasmic domains of the ErbB-2, termined NEC (Val.sup.664), or the point-mutated cytoplasmic domain of ErbB-2 (Glu.sup.664) termined TEC, (kindly obtained from Dr. Y. Yarden (Department of Chemical Immunology, the Weizmann Institute of Science, Rehovot, Israel); Peles, E., et al., J. Biol. Chem., 267:12266-12274 (1992)) were used in this study. It is Important to note that ErbB-2 does not directly bind to any of the EGF-like ligands. However, EGF and HRG induce the tyrosine phosphorylation of ErbB-2, presumably by ligand-driven heterodimerization and transphosphorylation. NIH3T3 cells were stably transfected with the chimeric plasmid EGF-TEC-ErbB-2 or with the chimeric plasmid EGF-NEC-ErbB-2. TEC and NEC cells (4.times.10.sup.6 cells/plate) were serum-starved and then unstimulated or stimulated with 100 ng EGF at room temperature for 5 minutes. The lysates were divided to two parts: one half of the lysates were precipitated with the CHK-SH2 GST-fusion protein (10 .mu.g) for 90 minutes at 4.degree. C. (A-II, B-II). After washing, the precipitates were separated by 7% SDS-PAGE and immunoblotted with monoclonal anti-phosphotyrosine antibody (PY20), or with polyclonal anti-EGF-R antibodies. The other half of the lysates was immunoprecipitated using monoclonal antibodies for EGF-R for 16 h at 4.degree. C. The washed precipitates were run on 7% SDS-PAGE and blotted with PY20 or with anti-EGF-R antibodies.

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L10: Entry 2 of 2 File: USPT Jan 25, 2000

US-PAT-NO: 6017527
DOCUMENT-IDENTIFIER: US 6017527 A

TITLE: Activated dendritic cells and methods for their activation

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maraskovsky; Eugene	Seattle	WA		
Mc Kenna; Hilary J.	Seattle	WA		

US-CL-CURRENT: 424/93.71; 424/93.7, 435/2, 435/325, 435/375, 435/377, 435/455

CLAIMS:

What is claimed is:

1. A method of stimulating an immune response specific for an antigen in an individual, comprising the steps of:

(a) obtaining dendritic cells from the individual;

(b) transfecting the dendritic cells with a gene encoding the antigen;

(c) activating the transfected dendritic cells by exposing them to a CD40 binding protein capable of binding CD40 and inhibiting binding of CD40 to CD40L, as determined by observing at least about 90% inhibition of the binding of soluble CD40 to CD40L; and,

(d) administering the activated, antigen-expressing dendritic cells to the individual.

2. The method according to claim 1, wherein the dendritic cells are obtained by obtaining hematopoietic stem or progenitor cells from the individual, and contacting the hematopoietic stem or progenitor cells with a molecule selected from the group consisting of flt-3 ligand, GM-CSF, IL-4, TNF-.alpha., IL-3, c-kit ligand, fusions of GM-CSF and IL-3, and combinations thereof.

3. The method according to claim 2, wherein the CD40 binding protein comprises an oligomer forming peptide and soluble, oligomeric CD40 ligand, wherein the soluble, oligomeric CD40 ligand comprises a polypeptide selected from the group consisting of:

(a) a polypeptide comprising amino acids 1 through 261, 35 through 261, 34 through 225, 113 through 261, 113 through 225, 120 through 261, or 120 through 225 of SEQ ID NO:2;

(b) fragments of a polypeptide according to (a) that bind CD40; and

(c) polypeptides encoded by DNA which hybridizes to a DNA that encodes a peptide of (a) or (b), under stringent conditions (hybridization in 6.times. SSC at

63.degree. C. overnight; washing in 3.times. SSC at 55.degree. C.), and which bind to CD40.

4. The method according to claim 3, wherein the soluble, oligomeric CD40 ligand comprises a polypeptide is selected from the group consisting of:

(a) a polypeptide having an amino acid sequence as set forth in SEQ ID NO:2 wherein a cysteine at amino acid 194 is replaced with another amino acid; and

(b) a polypeptide that is a fragment of the polypeptide (a) that binds CD40; wherein the amino acid that is substituted for the cysteine at amino acid 194 is selected from the group consisting of tryptophan, serine, aspartic acid, and lysine.

5. The method according to claim 1, wherein flt-3 ligand is administered to the individual prior to obtaining the dendritic cells, to expand the number of progenitor cells in the circulation of the individual.

6. The method according to claim 5, wherein the dendritic cells are obtained by obtaining hematopoietic stem or progenitor cells from the individual, and contacting the hematopoietic stem or progenitor cells with a molecule selected from the group consisting of flt-3 ligand, GM-CSF, IL-4, TNF-.alpha., IL-3, c-kit ligand, fusions of GM-CSF and IL-3, and combinations thereof.

7. The method according to claim 1, wherein the activated dendritic cells are administered simultaneously, sequentially or separately with a molecule selected from the group consisting of Interleukins 1, 2, 3, 4, 5, 6, 7, 10, 12 and 15; granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor; a fusion protein comprising Interleukin-3 and granulocyte-macrophage colony stimulating factor; Interferon-.gamma.; TNF; TGF-.beta.; flt-3 ligand; soluble CD40 ligand; soluble CD83; and combinations thereof.

8. The method according to claim 5, wherein the CD40 binding protein comprises an oligomer forming peptide and soluble, oligomeric CD40 ligand, wherein the soluble, oligomeric CD40 ligand comprises a polypeptide selected from the group consisting of:

(a) a polypeptide comprising amino acids 1 through 261, 35 through 261, 34 through 225, 113 through 261, 113 through 225, 120 through 261, or 120 through 225 of SEQ ID NO:2;

(b) fragments of a polypeptide according to (a) that bind CD40;

(c) polypeptides encoded by DNA which hybridizes to a DNA that encodes a polypeptide of (a) or (b), under stringent conditions (hybridization in 6.times. SSC at 63.degree. C. overnight; washing in 3.times. SSC at 55.degree. C.), and which bind to CD40;

(d) a polypeptide according to (a) wherein the cysteine at amino acid 194 is replaced with another amino acid selected from the group consisting of tryptophan, serine, aspartic acid, and lysine; and

(e) a fragment of the polypeptide of (d) which binds CD40.

9. The method according to claim 6, wherein the CD40 binding protein comprises an oligomer forming peptide and soluble, oligomeric CD40 ligand, wherein the soluble, oligomeric CD401 ligand comprises a polypeptide selected from the group consisting of:

(a) polypeptide comprising amino acids 1 through 261, 35 through 261, 34 through 225, 113 through 261, 113 through 225, 120 through 261, or 120 through 225 of SEQ ID NO:2;

(b) fragments of a polypeptide according to (a) that bind CD40;

(c) polypeptides encoded by DNA which hybridizes to a DNA that encodes a peptide of (a) or (b), under stringent conditions (hybridization in 6.times. SSC at 63.degree. C. overnight; washing in 3.times. SSC at 55.degree. C.), and which bind to CD40;

(d) a polypeptide according to (a) wherein the cysteine at amino acid 194 is replaced with another amino acid selected from the group consisting of tryptophan, serine, aspartic acid, and lysine; and

(e) a fragment of the polypeptide of (d) which binds CD40.

10. The method according to claim 7, wherein the CD40 binding protein comprises an oligomer forming peptide and soluble, oligomeric CD40 ligand, wherein the soluble, oligomeric CD40 ligand comprises a polypeptide selected from the group consisting of:

(a) a polypeptide comprising amino acids 1 through 261, 35 through 261, 34 through 225, 113 through 261, 113 through 225, 120 through 261, or 120 through 225 of SEQ ID NO:2;

(b) fragments of a polypeptide according to (a) that bind CD40;

(c) polypeptides encoded by DNA which hybridizes to a DNA that encodes a polypeptide of (a) or (b), under stringent conditions (hybridization in 6.times. SSC at 63.degree. C. overnight; washing in 3.times. SSC at 55.degree. C.), and which bind to CD40;

(d) a polypeptide according to (a) wherein the cysteine at amino acid 194 is replaced with another amino acid selected from the group consisting of tryptophan, serine, aspartic acid, and lysine; and

(e) a fragment of the polypeptide of (d) which binds CD40.

11. A method for stimulating an antigen-specific immune response comprising the steps of:

(a) obtaining hematopoietic stem or progenitor cells from an individual;

(b) obtaining dendritic cells by contacting, ex vivo, the cells of step (a) with cytokines suitable for culturing dendritic cells;

(c) exposing the dendritic cells of step (b) with a gene encoding an antigen, so as to obtain antigen-expressing dendritic cells;

(d) exposing the antigen-expressing dendritic cells of step (c) to a protein which binds CD40 and inhibits binding of CD40 to CD40L, so as to obtain activated, antigen-expressing dendritic cells; and

(e) administering an effective amount of the activated antigen-expressing dendritic cells of step (d) to the individual so as to stimulate the antigen-specific immune response in an individual.

12. The method according to claim 11, wherein the binding protein is selected from the group consisting of:

(a) a soluble, oligomeric CD40 ligand comprising amino acids 1-261 of SEQ ID NO:2 and an oligomer-forming peptide;

(b) a soluble, oligomeric CD40 ligand comprising amino acids 35-261 of SEQ ID NO:2 and an oligomer-forming peptide;

(c) a soluble, oligomeric CD40 ligand comprising amino acids 34-225 of SEQ ID NO:2 and an oligomer-forming peptide;

(d) a soluble, oligomeric CD40 ligand comprising amino acids 113-261 of SEQ ID

NO:2 and an oligomer-forming peptide;

(e) a soluble, oligomeric CD40 ligand comprising amino acids 113-225 of SEQ ID NO:2 and an oligomer-forming peptide;

(f) a soluble, oligomeric CD40 ligand comprising amino acids 120-261 of SEQ ID NO:2 and an oligomer-forming peptide;

(g) a soluble, oligomeric CD40 ligand comprising amino acids 120-225 of SEQ ID NO:2 and an oligomer-forming peptide; and

(h) fragments of a polypeptide according to (a)-(g) that bind CD40.

13. The method of claim 12 wherein the oligomer-forming peptide is an immunoglobulin heavy chain encoded by nucleotides 1-740 of SEQ ID NO:3.

14. The method of claim 12 wherein the oligomer-forming peptide is a leucine zipper represented by amino acids 1-33 of SEQ ID NO:4.

15. The method of claim 11 wherein the cysteine at amino acid 194 of SEQ ID NO:2 is substituted with an amino acid selected from the group consisting of tryptophan, serine, aspartic acid and lysine.

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L9: Entry 13 of 14 File: USPT Nov 2, 1999

US-PAT-NO: 5976546
DOCUMENT-IDENTIFIER: US 5976546 A

TITLE: Immunostimulatory compositions

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laus; Reiner	San Carlos	CA		
Ruegg; Curtis Landon	San Carlos	CA		
Wu; Hongyu	Palo Alto	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Dendreon Corporation	Seattle	WA			02

APPL-NO: 09/ 146283 [PALM]
DATE FILED: September 3, 1998

PARENT-CASE:
The application is a divisional of U.S. patent application Ser. No. 08/579,823 filed Dec. 28, 1995, now pending.

INT-CL: [06] A61 K 39/00, A61 K 39/385, C07 K 1/00, C07 K 14/00

US-CL-ISSUED: 424/192.1; 424/193.1, 424/194.1, 424/195.11, 530/350, 530/351
US-CL-CURRENT: 424/192.1; 424/193.1, 424/194.1, 424/195.11, 530/350, 530/351

FIELD-OF-SEARCH: 424/192.1, 424/193.1, 424/194.1, 424/195.11, 530/350, 530/351

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5616477	April 1997	Price	

ART-UNIT: 165

PRIMARY-EXAMINER: Caputa; Anthony C.

ASSISTANT-EXAMINER: Navarro; Mark

ATTY-AGENT-FIRM: Judge; Linda R. Dehlinger & Associates

ABSTRACT:

Disclosed are therapeutic compositions and methods for inducing cytotoxic T cell responses in vitro and in vivo. The therapeutic compositions consist of antigen presenting cells activated by contact with a polypeptide complex constructed by joining together a dendritic cell-binding protein and a polypeptide antigen. Also disclosed are expression vectors and systems for producing the polypeptide complexes.

2 Claims, 9 Drawing figures

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L9: Entry 13 of 14 File: USPT Nov 2, 1999

DOCUMENT-IDENTIFIER: US 5976546 A
TITLE: Immunostimulatory compositions

Brief Summary Text (14):
These limitations are overcome by the discovery encompassed by the present invention. It is the discovery of the present invention that a T-cell response, and specifically, an MHC-Class I mediated T-cell response, can be stimulated by an isolated or soluble protein, when it is presented to the immune system as part of a complex with a dendritic cell binding protein, and more particularly GM-CSF. It is the further discovery of the present invention that such a response can be stimulated in vitro. As discussed above, in vitro stimulation of such a response has not previously been demonstrated using a full-length soluble antigen. The present invention provides for induction by isolated or soluble proteins of cellular immunity in vitro by presenting a specific antigen to an antigen presenting cell (APC), such as a dendritic cell, as part of an immunogenic fusion protein.

Brief Summary Text (15):
An important aspect of the present invention is the choice of fusion partner protein, a dendritic cell binding protein, such as granulocyte-macrophage colony stimulating protein (GM-CSF). Without relying on any particular mechanistic theory, it is believed that the protein antigen is transported over the plasma membrane of the APC in a receptor mediated non-disruptive way. It is further believed that the dendritic cell binding portion of the fusion protein serves to preserve the viability and functionality of the APC.

Detailed Description Text (37):
The use as APC's of dendritic cells stimulated by GM-CSF fusion antigens yields superior results to other approaches such as peptide pulsed dendritic cells. It is known that dendritic cells pulsed with 8-11-mer peptides induce immunity that is directed to a single T-cell epitope. Proteins incorporated into liposomes or delivered by osmotic shock induce reactivity towards multiple T-cell epitopes; however, this process is relatively ineffective due to the inherent toxicity of these treatments to dendritic cells. GM-CSF fusion antigens of the present invention, on the other hand, induce immunity towards multiple epitopes and preserve and enhance at the same time viability and function of the dendritic cell.

Detailed Description Text (39):
In experiments carried out in support of the present invention, the fusion protein consisting of PAP and GM-CSF described in the previous section was used for in vitro introduction to dendritic cells and subsequent activation of cytolytic T cells, as detailed in Example 4. Briefly, HLA-A2.1 positive PBMNC were isolated by standard methods and primed with the fusion protein for 2-5 days. The cell mixture was depleted of CD4.sup.+ T-cells, separated into high and low density fractions, and the separate cultures were restimulated weekly with autologous PAP GM-CSF pulsed APC's. Lytic potential of the T-cells present in the fractions was assessed using a standard chromium release assay using an HLA-A2.1-transgenic prostate carcinoma cell line as target. This novel cell line was constructed according to the methods detailed in Example 3 herein and is useful in screening and analysis of HLA class I restricted cytotoxic T-lymphocytes.

Detailed Description Text (46):
In practice, dendritic cells are isolated from an individual, using known methods, or preferably, as described in Example 5. The dendritic cells (or other APC's) are mixed

with 10 ng/ml equivalent of GM-CSF fusion antigen, as described in Example 4. The cell preparation may then be depleted of CD4.sup.+ T-cells by solid phase immunoadsorption and further fractionated to enrich for cells having cytolytic activity. Doses of about 10.sup.7 cells are then administered to the subject by intravenous or central injection according to established procedures (e.g., infusion over 30 to 60 minutes). The responsiveness of the subject to this treatment is measured by monitoring the induction of a cytolytic T-cell response, a helper T-cell response and antibody response towards the antigen in peripheral blood mononuclear cells by methods well known in the art.

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L5: Entry 10 of 17 File: USPT Apr 3, 2001

US-PAT-NO: 6210662
DOCUMENT-IDENTIFIER: US 6210662 B1

102e ref

TITLE: Immunostimulatory composition

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laus; Reiner	San Carlos	CA		
Ruegg; Curtis Landon	San Carlos	CA		
Wu; Hongyu	Palo Alto	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Dendreon Corporation	Seattle	WA			02

APPL-NO: 09/ 344195 [PALM]
DATE FILED: June 24, 1999

PARENT-CASE:
This application is a continuation of U.S. patent application Ser. No. 09/146,283, filed Sep. 3, 1998, now granted as U.S. Pat. No. 5,976,546, which is a divisional of U.S. patent application Ser. No. 08/579,823, filed Dec. 28, 1995 now granted as U.S. Pat. No. 6,080,409, both of which are herein incorporated by reference.

INT-CL: [07] A01 N 63/00, A01 N 65/00, C12 N 5/00, C12 N 5/08

US-CL-ISSUED: 424/93.1; 435/325, 435/366, 435/372, 435/372.3
US-CL-CURRENT: 424/93.1; 435/325, 435/366, 435/372, 435/372.3

FIELD-OF-SEARCH: 424/93.1, 435/325, 435/366, 435/372, 435/372.3

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

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☐	5019384	May 1991	Gefter et al.	
☐	5078996	January 1992	Colon, III et al.	
☐	5194254	March 1993	Barber et al.	
☐	5616477	April 1997	Price	
☐	5677171	October 1997	Hudziak et al.	

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FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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WO 91/13632	September 1991	WO	
WO 93/20185	October 1993	WO	
WO 94/17192	August 1994	WO	
WO 94/21287	September 1994	WO	
WO 95/21862	August 1995	WO	
WO 95/23814	September 1995	WO	
WO 95/34638	December 1995	WO	

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ART-UNIT: 165

PRIMARY-EXAMINER: Navarro; Albert

ATTY-AGENT-FIRM: Judge; Linda R. Iota Pi Law Group

ABSTRACT:

Disclosed are therapeutic compositions and methods for inducing cytotoxic T cell responses in vitro and in vivo. The therapeutic compositions consist of antigen presenting cells activated by contact with a polypeptide complex constructed by joining together a dendritic cell-binding protein and a polypeptide antigen. Also disclosed are expression vectors and systems for producing the polypeptide complexes.

3 Claims, 9 Drawing figures

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L5: Entry 10 of 17 File: USPT Apr 3, 2001

DOCUMENT-IDENTIFIER: US 6210662 B1
TITLE: Immunostimulatory composition

Brief Summary Text (23):
In further related aspects, the invention also includes expression vectors and expression systems for producing the above-described immunostimulatory fusion proteins, as well as substantially purified nucleic acid molecules that encode such fusion proteins. In preferred embodiments, the nucleic acid molecules code for fusion proteins consisting essentially of GM-CSF and prostatic acid phosphatase or of GM-CSF and Her2.

Drawing Description Text (9):
FIG. 8 shows the nucleic acid (SEQ ID NO: 3) and amino acid (SEQ ID NO: 4) sequences of a GM-CSF-Her2 fusion protein in accordance with the present invention.

Detailed Description Text (32):
Fusion proteins constructed to incorporate oncogene product antigens are exemplified by incorporation of the oncogene product Her2. Her2 is a growth factor receptor belonging to the EGF-R family of receptors. It is overexpressed by breast cancer cells, ovarian cancer cells and a variety of other cancer cells. The cDNA coding for the extracellular domain of Her2 is cloned from a breast cancer cell line and fused to the GM-CSF CDNA, essentially as detailed for PAP-GM-CSF, above.

Detailed Description Text (33):
Production of the soluble protein can be verified using Her2-specific monoclonal antibodies in an ELISA test, according to methods well-known in the art. The fusion protein the includes the sequences for the extracellular domain (amino acids 1-652) of Her2 (GenBank) and GM-CSF (FIG. 8). In this particular fusion protein the two proteins are linked by a leucine/glutamic acid linker which is generated by inserting a XhoI site. Other oncogene product antigens are similarly incorporated into fusion proteins according to the methods described herein, using published sequences. In addition, other antigens, such as viral antigens, may be part of a fusion construct, according to the methods described herein.

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FD 10/18/00

L8: Entry 7 of 11 File: USPT Apr 8, 2003

DOCUMENT-IDENTIFIER: US 6544518 B1
TITLE: Vaccines

Brief Summary Text (97):
The HER-2/neu PD is 268 amino acids in length, is intracellular, and can be phosphorylated by protein tyrosine kinases. The region shares no identity with the corresponding part of other tyrosine kinase receptors. Thus, the specificity and uniqueness of this domain makes it particularly preferred for use as a tumour vaccine. However, the expression of this domain alone in bacterial and mammalian cells is problematic. For example, the resultant PD protein is very labile and is not appropriate for large scale production. In one embodiment, this invention thus preferably utilises a fusion comprising all or part of the intracellular domain or the phosphorylation domain to all or part of the HER-2/neu extracellular domain. The ECD-ICD fusion proteins and the ECD-PD fusion proteins of the invention are soluble. are secreted and are stable in culture media.

Brief Summary Text (98):
The vaccines of the invention will be useful against any cancer characterised by tumour associated antigen expresion, such as HER-2/neu expression. In addition to allowing increased expression of the intracellular domain or phosphorylation domain, or variants thereof, as a fusion protein with the extracelluar domain or its variants, the ECD-ICD and ECD-PD fusion proteins provide for an improved vaccine formulation.

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L22: Entry 21 of 29 File: USPT Apr 8, 2003

DOCUMENT-IDENTIFIER: US 6544518 B1
TITLE: Vaccines

Brief Summary Text (91):
The Her2-neu antigen may be the entire Her2-neu antigen or portions thereof. Preferred portions comprises the extracellular domain. In a more preferred embodiment there is provided an fusion protein comprising an extracellular domain linked to a portion of the intracellular domain as disclosed in WO 00/44899.

Brief Summary Text (94):
The "HER-2/neu ECD-ICD fusion protein," also referred to herein as "ECD-ICD" or "ECD-ICD fusion protein," refers to a fusion protein (or fragments thereof) comprising the extracellular domain (or fragments thereof) and the intracellular domain (or fragments thereof) of the HER-21neu protein. These represent preferred antigens to utilise in the context of the present invention. As used herein, the ECD-ICD fusion protein does not include a substantial portion of the HER-2/neu transmembrane domain, and preferably does not include any of the HER-2/neu transmembrane domain.

Brief Summary Text (95):
The "HER-2/neu ECD-PD fusion protein,>also referred to as "ECD-PD" or "ECD-PD fusion protein," or the "HER-2/neu ECD-.DELTA.PD fusion protein," also referred to as "ECD-.DELTA.PD" or ECD-.DELTA.PD fusion protein," refer to fusion proteins (or fragments thereof) comprising the extracellular domain (or fragments thereof) and phosphorylation domain (or fragments thereof, eg, .DELTA.PD) of the HER-2/neu protein. The ECD-PD and ECD-.DELTA.PD fusion proteins do not include a substantial portion of the HER-2/neu transmembrane domain, and preferably do not include any of the HER-2/neu transmembrane domain.

Brief Summary Text (97):
The HER-2/neu PD is 268 amino acids in length, is intracellular, and can be phosphorylated by protein tyrosine kinases. The region shares no identity with the corresponding part of other tyrosine kinase receptors. Thus, the specificity and uniqueness of this domain makes it particularly preferred for use as a tumour vaccine. However, the expression of this domain alone in bacterial and mammalian cells is problematic. For example, the resultant PD protein is very labile and is not appropriate for large scale production. In one embodiment, this invention thus preferably utilises a fusion comprising all or part of the intracellular domain or the phosphorylation domain to all or part of the HER-2/neu extracellular domain. The ECD-ICD fusion proteins and the ECD-PD fusion proteins of the invention are soluble. are secreted and are stable in culture media.

Brief Summary Text (98):
The vaccines of the invention will be useful against any cancer characterised by tumour associated antigen expresion, such as HER-2/neu expression. In addition to allowing increased expression of the intracellular domain or phosphorylation domain, or variants thereof, as a fusion protein with the extracelluar domain or its variants, the ECD-ICD and ECD-PD fusion proteins provide for an improved vaccine formulation.

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L1: Entry 12 of 12 File: USPT Jun 18, 2002

FD 8/21/00

DOCUMENT-IDENTIFIER: US 6406681 B1
TITLE: Method of treating a systemic disease

Detailed Description Text (22):
The term "vaccines" refers to therapeutic compositions for stimulating humoral and cellular immune responses, either isolated, or through an antigen presenting cell, such as an activated dendritic cell, that is able to activate T-cells to produce a multivalent cellular immune response against a selected antigen. The potent antigen presenting cell is stimulated by exposing the cell in vitro to a polypeptide complex. The polypeptide complex may comprise a dendritic cell-binding protein and a polypeptide antigen, but preferably, the polypeptide antigen is either a tissue-specific tumor antigen or an oncogene gene product. However, it is appreciated that other antigens, such as viral antigens can be used in such combination to produce immunostimulatory responses. In another preferred embodiment, the dendritic cell-binding protein that forms part of the immunostimulatory polypeptide complex is GM-CSF. In a further preferred embodiment, the polypeptide antigen that forms part of the complex is the tumor-specific antigen prostatic acid phosphatase. In still other preferred embodiments, the polypeptide antigen may be any one of the oncogene product peptide antigens. The polypeptide complex may also contain, between the dendritic cell-binding protein and the polypeptide antigen, a linker peptide. The polypeptide complex may comprise a dendritic cell-binding protein covalently linked to a polypeptide antigen, such polypeptide complex being preferably formed from a dendritic cell binding protein, preferably GM-CSF, and a polypeptide antigen. The polypeptide antigen is preferably a tissue-specific tumor antigen such as prostatic acid phosphatase (PAP), or an oncogene product, such as Her2, p21RAS, and p53; however, other embodiments, such as viral antigens, are also within the scope of the invention.

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L30: Entry 4 of 10 File: USPT Jun 13, 2000

US-PAT-NO: 6075122
DOCUMENT-IDENTIFIER: US 6075122 A

TITLE: Immune reactivity to HER-2/neu protein for diagnosis and treatment of malignancies in which the HER-2/neu oncogene is associated

DATE-ISSUED: June 13, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheever; Martin A.	Mercer Island	WA		
Disis; Mary L.	Renton	WA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
University of Washington	Seattle	WA			02

APPL-NO: 08/ 466680 [PALM]
DATE FILED: June 6, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of U.S. patent application Ser. No. 08/414,417, filed Mar. 31, 1995, U.S. Pat. No. 5,801,005; which application is a continuation-in-part application to Ser. No. 08/106,112, filed Aug. 12, 1993, abandoned; which application is a continuation-in-part application to Ser. No. 08/033,644, filed Mar. 17, 1993, abandoned.

INT-CL: [07] C07 K 14/47, C07 K 14/705

US-CL-ISSUED: 530/350; 530/806, 530/828
US-CL-CURRENT: 530/350; 530/806, 530/828

FIELD-OF-SEARCH: 530/300, 530/327, 530/328, 530/350, 530/806, 530/828, 530/326

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	3766162	October 1973	Spector	
☐	3791932	February 1974	Schuurs et al.	
☐	3817837	June 1974	Rubenstein et al.	
☐	3850752	November 1974	Schuurs et al.	
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☐	3935074	January 1976	Rubenstein et al.	
☐	3984533	October 1976	Uzgiris	
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ART-UNIT: 162

PRIMARY-EXAMINER: Johnson; Nancy A.

ATTY-AGENT-FIRM: Seed and Berry LLP

ABSTRACT:

Methods for the detection, monitoring and treatment of malignancies in which the HER-2/neu oncogene is associated are disclosed. Detection of specific T cell activation (e.g., by measuring the proliferation of T cells) in response to in vitro exposure to the HER-2/neu protein, or detection of immunocomplexes formed between the HER-2/neu protein and antibodies in body fluid, allows the diagnosis of the presence of a malignancy in which the HER-2/neu oncogene is associated. The present invention also discloses methods and compositions, including peptides, for treating such malignancies.

2 Claims, 24 Drawing figures

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L30: Entry 4 of 10 File: USPT Jun 13, 2000

DOCUMENT-IDENTIFIER: US 6075122 A
TITLE: Immune reactivity to HER-2/neu protein for diagnosis and treatment of malignancies in which the HER-2/neu oncogene is associated

FD = 6/6/95

INVENTOR (1):
Cheever; Martin A.

Drawing Description Text (8):
FIG. 7 pictorially illustrates that some breast cancer patients have antibodies directed to both the extracellular and intracellular domain of the HER-2/neu protein. Sera of breast cancer patients is tested against the extracellular domain ("ECD protein") or the intracellular domain ("ICD protein"), in lanes A and B, respectively.

Detailed Description Text (39):
Peptides, each 15 amino acids in length, that encompass both the AMPHI and Rothbard motifs were constructed. The optimal peptide length for class II MHC binding depends upon the particular MHC molecule and may be shorter than 15 amino acids. However, class II MHC responses to exogenous peptides allow for endocytosis and intracellular processing of longer peptides. One of the synthetic peptides (p42-56), HLDMLRHLYQGCQVV (Seq. ID No. 30), is located in the extracellular domain and has 33% homology to epidermal growth factor receptor (EGFR). Two other synthetic peptides, SRLLGICLTSTVQLV (p783-797) (Seq. ID No. 45) and TLERPKTLSPGKNGV (p1166-1180) (Seq. ID No. 54)are both located in the intracellular domain and have 87% and 7% homology to EGFR respectively. The peptides as well as partially purified whole protein (p185.sup.HER-2/neu) were used in subsequent defined experiments to detect CD4+ T cell proliferation responses (Section C below).

Check- ① conjugated to cancer? ② fusion? ③ to cytokine, GMCSF?

Detailed Description Text (60):
According to the current motif, the p185.sup.HER-2/neu protein contains a substantial number of peptides with amino acid sequences possibly appropriate for binding to the class I MHC antigen HLA-A2.1. Evaluation of the 1255 aa structure of p185.sup.HER-2/neu revealed at least 19 peptide segments of 9 aa in length that contained at least one of the dominant anchor residues. Of note, the current HLA-A2.1 motif places 6 amino acids between the dominant anchor amino acids at residues 2 and 9. Recent studies show that alterations in secondary structure of peptides can sometimes allow for additional intervening residues, and thus longer binding peptides. In the present experiment, 9-mer peptides were evaluated. The 10 peptides with both dominant residues were considered. The arbitrary scoring system awarded +3 for a dominant anchor residue, +2 for a strong binding residue, and +1 for a weak binding residue. Emphasis was placed on presence or absence of dominant anchor residues as they appear to be of prime importance for peptide binding to HLA-A2 (Parker et al., J. Immunol. 148:3580-3587, 1992). Four peptides were synthesized (Table 3). One is located in the extracellular domain of the protein and three are located in the intracellular domain. Homology to EGFR ranges from 11% to 89% (Bargmann et al., Nature 319:226-230, 1986).

Detailed Description Text (67):
Initial experiments examined response to p48-56 which is normally present in the extracellular domain and p789-797 which is normally present in the intracellular domain, both of which were found to bind to HLA-A2.1. Four of four peptides with a motif theoretically appropriate for binding to HLA-A2.1 are shown to actually bind to HLA-A2.1 in a class I MHC molecule stabilization assay (Table 4). T2 cells were

incubated for 18 hours with the depicted synthetic p185.sup.HER-2/neu peptides. Cells were then washed and stained with antihuman HLA-A2 antibody (3%), a second step FITC-conjugated antibody (3%) was then added. The % increase of class I on cell surface as measured by increased fluorescent intensity of cells incubated with peptide compared to cells incubated in medium alone is indicated.

Detailed Description Text (76):
Recombinant proteins of the extracellular and intracellular domain portions of HER-2/neu were obtained. The extracellular protein (110kD) and intracellular protein (75 kD) were resolved on a 7.5%; SDS-PAG gel and incubated with patient sera as primary antibody as previously described. The sera identified both proteins proving that some patients have antibodies directed to both the extracellular and intracellular domain of the HER-2/neu protein (FIG. 7).

Detailed Description Text (90):
One group of rats was immunized with a mixture of extracellular domain (ECD) peptides and one group with a mixture of intracellular domain (ICD) peptides. The 15 final group received adjuvant alone. Peptides were administered at a final concentration of 100 .mu.g each in a total volume of 200 .mu.l. The animals underwent 3 immunizations each 14-16 days apart with either CFA or IFA as adjuvant (Sigma ImmunoChemicals, St. Louis, Mo.). 16 days after the third immunization sera was obtained for assessment of immune response.

Detailed Description Paragraph Table (6):
TABLE 3
p185.sup.HER-2/neu Peptides Constructed for Binding in HLA-A2.1 Motif
p185.sup.HER-2/neu Amino Acid Position Homology Peptides 1 2 3 4 5 6 7 8 9 Score Location to EGFR

p48-56.sup.HER-2/neu H L Y Q G C Q V V 8* Extracellular 33% (Seq. ID No. 1)
p789-797.sup.HER-2/neu C L T S T V Q L V 9* Intracellular 89% (Seq. ID No. 7)
p851-859.sup.HER-2/neu V L V K S P N H V 9* Intracellular 78% (Seq. ID No. 9)
p1172-1180.sup.HER-2/neu T L S P G K N G V 9* Intracellular 11% (Seq. ID No. 10)
*Peptide contains both dominant anchor residues

Detailed Description Paragraph Table (8):
TABLE 5 Peptides from the Rat neu Protein for Immunization Homology Rat Protein to Human Sequence Amino Acids Domain neu
p45-59 HLDMLRHLYQGCQVV ECD 100% (Seq. ID No. 30) p98-112 PLQRLRIVRGTQLFE ECD 100% (Seq. ID No. 31) p323-337 NQEVTAEDGTQRCEK ECD 100% (Seq. ID No. 56) p332-349 TQRCEKCSKPCARVCYGL ECD 100% (Seq. ID No. 60) p433-447 RIIRGRILHDGAYSL ECD 80% (Seq. ID No. 67) p781-795 GVGSPYVSRLLGICL ICD 100% (Seq. ID No. 44) p788-802 SRLLGICLTSTVQLV ICD 100% (Seq. ID No. 45) p932-946 PAREIPDLLEKGERL ICD 100% (Seq. ID No. 49) p1171-1185 TLERPKTLSPGKNGV ICD 100% (Seq. ID No. 54)
ECD = extracellular domain ICD = intracellular domain

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L23: Entry 34 of 43 File: USPT Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333169 B1
**** See image for Certificate of Correction ****
TITLE: HER2 extracellular domain

Abstract Text (1):
An extracellular portion of the HER2 molecule, essentially free of transmembrane and cytoplasmic portions, which is antigenic in animals. Isolated DNA encoding the extracellular portion; an expression vector containing the isolated DNA; and a cell containing the expression vector. A process for producing the extracellular domain. A vaccine containing the extracellular domain.

Brief Summary Text (18):
In a yet further embodiment, the present invention extends to a vaccine comprising the extracellular portion of the HER2 molecule, which may be combined with suitable adjuvants.

Detailed Description Text (40):
In addition to Active Specific Immunotherapy, it should be possible to use the purified extracellular domain to isolate and characterize the putative ligand. The HER2 ligand may be used in turn to deliver toxin to tumor cells which are overexpressing HER2, such as by molecular fusion of the ligand with toxin, or by chemical cross-linking. Alternatively, patients overexpressing HER2 may be vaccinated with HER2 ligand conjugated to, or in combination with, a suitable adjuvant.

Other Reference Publication (22):
Fendly et al., "Successful Immunization of Rhesus Monkeys with Extracellular Domain of p185.sup.HER2 :A Potential Approach to Human Breast Cancer" Vaccine Research 2(3):129-139 (1993).

Other Reference Publication (58):
Ezzell, C., "Cancer `Vaccines`: An Idea Whose Time Has Come?" Journal of NIH Research 7:46-49 (Jan. 1995).

CLAIMS:

4. A method for making a polypeptide fusion comprising an extracellular domain of the HER2 receptor conjugated to a Fc portion of an immunoglobulin molecule, comprising:

(a) culturing a host cell comprising DNA encoding said polypeptide fusion under conditions suitable for expression of said DNA; and

(b) recovering said polypeptide fusion from said host cell.

8. A method for making a vaccine useful for Active Specific Immunotherapy, comprising:

(a) culturing a host cell comprising DNA encoding the extracellular domain of the HER2 receptor or a portion thereof which provokes a humoral and cell-mediated response against HER2 receptor in a patient vaccinated therewith under conditions suitable for expression of said DNA;

(b) recovering said extracellular domain or portion thereof from said host cell; and

(c) combining said recovered extracellular domain or portion thereof with an adjuvant which is safe and effective in humans so as to generate a vaccine which provokes a humoral and cell-mediated response against HER2 receptor in a patient vaccinated therewith.

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End of Result Set

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L21: Entry 3 of 3 File: USPT Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333169 B1
**** See image for Certificate of Correction ****
TITLE: HER2 extracellular domain

CLAIMS:

4. A method for making a polypeptide fusion comprising an extracellular domain of the HER2 receptor conjugated to a Fc portion of an immunoglobulin molecule, comprising:

(a) culturing a host cell comprising DNA encoding said polypeptide fusion under conditions suitable for expression of said DNA; and

(b) recovering said polypeptide fusion from said host cell.

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1: Int Immunopharmacol. 2002 May;2(6):783-96. Related Articles, Links

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Efficacy of vaccination with plasmid DNA encoding for HER2/neu or HER2/neu-eGFP fusion protein against prostate cancer in rats.

Bhattachary R, Bukkapatnam R, Prawoko I, Soto J, Morgan M, Salup RR.

University of South Florida, Tampa 33620-5950, USA. rbhattac@com1.med.usf.edu

Despite early diagnosis and improved therapy, 31,500 men will die from prostate cancer (PC) this year. The HER2/neu oncoprotein is an important effector of cell growth found in the majority of high-grade prostatic tumors and is capable of rendering immunogenicity. The antigenicity of this oncoprotein might prove useful in the development of PC vaccines. Our goal is to prove the principle that a single DNA vaccine can provide reliable immunity against PC in the MatLyLu (MLL) translational tumor model. The parental rat MatLyLu PC cell line expresses low to moderate levels of the rat neu protein. To simulate in vivo human PC, MatLyLu cells were transfected with a truncated sequence of human HER2/neu cDNA cloned into the pCI-neo vector. This HER2/neu cDNA sequence encodes the first 433 amino acids of the extracellular domain (ECD). MatLyLu cells were also transfected with the same HER2/neu cDNA sequence cloned into the N1-terminal sequence of EGFP reporter gene to produce a fusion protein. The partial ECD sequence of HER2/neu includes five rat major histocompatibility (MHC)-II-restricted peptides with complete human-to-rat cross-species homology. The HER2/neu protein overexpression was documented by Western Blot analysis, and the expression of fusion protein was monitored by confocal microscopy and fluorimetry. Vaccination with a single injection of HER2/neu cDNA protected 50% of animals against HER2/neu-MatLyLu tumors ($P < 0.01$). When the tumor cells were engineered to express HER2/neu-EGFP fusion protein, the antitumor immunity was enhanced, as following vaccination with HER2/neu-EGFP cDNA, 80% of these rats rejected HER2/neu-EGFP-MatLyLu ($P<0.001$). Both vaccines induced HER2/neu-specific antibody titers. Rats vaccinated with EGFP-cDNA rejected 80% of EGFP-MatLyLu tumors and, interestingly, 40% of HER2/neu-MatLyLu tumors. None of the cDNA vaccines induced immunity against parental MatLyLu cells. Our data clearly demonstrate that a single injection of HER2/neu-EGFP cDNA is a very effective vaccine against PC tumors expressing the cognate tumor-associated antigen (TA). The antitumor immunity is significantly more pronounced if the tumors express xenogeneic HER2/neu-EGFP fusion protein as opposed to only the syngeneic HER2/neu oncoprotein. Our data suggests that the HER2/neu-EGFP-MatLyLu tumor is a potential animal tumor model for investigating therapeutic vaccine

strategies against PC in vivo and demonstrates the limitations of a cDNA vaccine only encoding for MHC-II-restricted HER2/neu-ECD sequence peptides.

PMID: 12095169 [PubMed - indexed for MEDLINE]

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